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Marked intrafamilial variability of exocrine and endocrine pancreatic phenotypes due to a splice site mutation in *GATA6*

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ABSTRACT

The objective of this study was to describe the clinical characteristics of syndromic neonatal diabetes in a family with a *GATA6* mutation. A girl, currently aged 12 years 3 months, was born with intrauterine growth retardation: weight 1600 g (–4.3 SDS) at term. After birth, foramen ovale and patent ductus arteriosus (PDA) were diagnosed by echocardiography. Diabetes was diagnosed on the 9th day after birth. Exocrine pancreatic insufficiency was clinically diagnosed at about 2 years of age and pancreatic agenesis was revealed later by magnetic resonance imaging. Her father had undergone surgery during infancy for PDA and had developed insulin dependent diabetes at 12 years of age. Ultrasound revealed a thin pancreas with normal length and anatomical structure. He has subclinical exocrine pancreatic insufficiency, low insulin needs and no late complications of diabetes up to the age of 40 years. Sequencing of *GATA6* identified a heterozygous splicing mutation, 1136-2A>G, in the girl and her father. Testing of the paternal grandparents showed that the mutation was likely to have arisen *de novo* in the father. Identification of a *GATA6* mutation explains the cardiac anomalies and diabetes in this family. This case highlights the marked intrafamilial variability of both exocrine and endocrine pancreatic phenotypes in patients with *GATA6* mutations.

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Permanent neonatal diabetes mellitus; pancreatic agenesis; pancreatic hypoplasia; *GATA 6*; congenital cardiac defects; patent ductus arteriosus

Introduction

Genome sequencing technologies have helped to establish the etiology of diabetes mellitus (DM) in a small group of pediatric patients presenting non-autoimmune mechanisms of impaired insulin secretion. Some of them are diagnosed in the neonatal period or during early infancy. More than 20 gene mutations causing permanent (PNDM) or transient (TNDM) neonatal diabetes mellitus have been identified [1].

The transcription factor GATA6 has been intensively studied after the discovery of its role in the development of the pancreas. GATA 4/5/6 subfamily presents primary endodermal and mesodermal transcription factors, expressed in the primitive endoderm and extra-embryonic tissues for supporting the growing epiblast [2–4] and endodermal differentiation [5]. During organogenesis, GATA4/5/6 ensure the anatomical and functional development of the pancreas, heart, blood vessels, hepatobiliary system, urogenital tract, thyroid gland as well as the brain [6,7]. GATA 4/6 have a leading role in the transcriptional network hierarchy in pancreatic multipotent progenitors arisen from the foregut endoderm [8,9].

In humans, *GATA4* mutations were initially discovered to cause familial congenital cardiac septal defects [10] and have been found in some individuals from screened series of patients with congenital non-syndromic cardiac defects.[11–13] Further studies have shown that *GATA4/6* haploinsufficiency can also cause a combination of pancreatic agenesis/hypoplasia, cardiac and hepatobiliary defects [14–16].

In this report we describe the clinical characteristics of syndromic neonatal diabetes in a family, father and daughter, with a *GATA6* mutation. The genetic finding has already been reported [16]. Here, the clinical features are described in details and, especially, the marked phenotypic variability between the two affected family members.

Subjects and methods

This study included two family members, father and daughter, reportedly carrying a *GATA6* mutation [16]. The girl patient, currently aged 12 years 3 months was born with intrauterine growth retardation with weight

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1600 g (−4.3 SDS), length 41 cm (−4.8 SDS), following a normal full-term pregnancy of a healthy mother and a father diagnosed with type 1 diabetes at the age of 12 years.

Written informed consent to participate in this study was obtained for both subjects. The study was approved by the Ethics committee at the University Pediatric Hospital–Sofia.

Genetic testing

Molecular genetic analysis was performed by screening the coding sequence and ~50 bp of flanking sequence of *GATA6* using Sanger sequencing [16]. Sequencing reactions were run on an ABI3730 capillary machine (Applied Biosystems, Warrington, U.K.) and analysed using Mutation Surveyor v3.98 (SoftGenetics, State College, PA) (GATA6 nucleotide reference NM_005257.3).

Results and discussion

Clinical case

The first ultrasound cardiac investigation of the girl revealed foramen ovale, small patent ductus arteriosus (PDA) and peripheral stenosis of both branches of the pulmonary artery. At the age of 9 days, her condition worsened with dehydration, weight loss up to 1200 g, hyperglycemia of 30 mmol/L without ketonuria and hyponatriemia 128 mmol/L (range 130–155). Intensive insulin replacement therapy was started with diluted short-acting human insulin at a daily dose of 0.05 U/kg body weight (b.w.). Anti-GAD65, IAA and IA2 antibodies were all negative. At the age of 20 days, C-*p*eptide was shown to be deficient (73.1 pmol/L; range 174–960 pmol/L).

The father was born with normal birth weight. He underwent a cardiac operation in infancy for PDA and developed diabetes at the age of 12 years. He has always been with mild insulin requirements, treated with two injections of mixed insulin at a daily dose 0.35 U/kg b.w. He had no severe exocrine pancreatic insufficiency proven by the following clinical evidence: no gastrointestinal problems in early childhood, normal physical development with height of 178 cm, normal body mass index, normal abdominal circumference, normal stools. He has normal intellectual development, secondary education and no late diabetes complications at the age of 40 years. In 2011, his examination revealed glycated hemoglobin (HbA1c) 7.9%/62.84 mmol/mol (normal 4%–6.2%/20.22–44.26 mmol/mol), normal hepatic function and lipid metabolism. C-peptide was low: 88.2 pmol/L (normal 196–960 pmol/L). Ultrasound investigation revealed a hypoplastic thin pancreas but normal length and anatomical structure. Fecal elastase measured at his last visit was less than 15 μg/g feces (normal $>$ 200 μg/g), suggesting subclinical pancreatic insufficiency.

Clinical course

In the girl, the ligation of PDA was performed at 20 months of age because of clinically significant signs of large left to right shunting, marked left ventricular volume overload and congestive heart failure. After surgery, the specific medical therapy was stopped and the cardiac dimensions gradually normalized. During the first 3 years of life, the main clinical issues were iron-deficient anemia, hypoproteinemia, mildly elevated transaminases and subclinical biliary stasis, perhaps all attributable to hepatic dysfunction and/or steatosis hepatitis due to some degree of insulin deficiency and malnutrition (Table 1).

Breast milk enriched in proteins and starch was given in the first few months after birth. Unusually large amount of carbohydrates, up to 20–22 g/kg b.w. (normal 12–14 g/kg), was necessary to keep weight gain with a corresponding insulin dose of 0.5 U/kg b.w. Follow-ups of C-peptide were recorded at 3 months (79.6 pmol/L) and 2 years (19.4 pmol/L). Clinical signs of exocrine pancreatic insufficiency appeared after 2 years of age: abdominal distension, bulky stools 5–7 times daily with steatorrhea with up to 50% of ingested fat. No weight loss was seen and the insulin dose was stable (0.8–0.9 U/kg b.w.). Unfortunately, we could not investigate fecal

Table 1. Laboratory signs of hepatic function.

Age	ASAT[a] U/L	ALAT[b] U/L	GGT[c] U/L	Alkaline phosphatase U/L	Total bilirubin (μmol/L) (n < 17)	Direct bilirubin (μmol/L) (n = 0–3.5)
3 months	76 (n < 61)	39 (n < 50)	316 (n = 4–163)	1626 (n = 124–341)	49.0	15.4
1.1 years	32 (n < 50)	42 (n < 36)	12 (5–31)	875 (108–317)		
2 years	73	73	12	876		
2.4 years	60	74	11	838		
3 years	182	117	14	323		
3.5 years	28	31	12	314		

[a]Alanine aminotransferase.
[b]Aspartate aminotransferase.
[c]Gamma-glutamyl transpeptidase.
Note: *n*, normal (reference) value.

elastase at the time. Ultrasound revealed initially a small pancreas with 3.5 mm length. Replacement therapy similar to that of patients with cystic fibrosis was initiated at age of 2 years 2 months: 1000 lipase units/kg b.w. per meal, Vitamin A 2000 U/d, Vitamin D 800 U/d and Vitamin E 50 U/d. At the age of 4 years, magnetic resonance imaging (MRI) was performed and confirmed pancreatic agenesis. Fecal elastase measured at her last visit was less than 15 μg/g feces (normal $>$ 200 μg/g).

Current status

The girl takes a normal traditional diet and receives three to four injections of short-acting insulin analog for meals and long-acting insulin analog at bedtime with a total daily dose of 0.8 U/kg b.w. The dose of Creon is 75,000–100,000 U daily. At the last examination, the plasma levels of fat-soluble vitamins under daily supplemental therapy were near the lower reference: Vitamin A, 0.24 mg/L (0.3–0.8 mg/L); Vitamin E (tocopherol), 7.9 mg/L (5–18 mg/L) and 25-OH-Vitamin D, 19.42 ng/mL (sufficiency level 30–100 ng/mL).

At present (12 years 3 months of age), the girl's height is 151.5 cm (−0.06 SDS), weight 37 kg (−0.83 SDS) (Figure 1). The expected final height according to the parent's height is 162.5 $\pm$ 5 cm.

She has normal pubertal (Tanner stage 3) and intellectual development with good school performance and normal physical activity. She maintains good glycemic control with HbA1c 7%–8% (53.1–63.93 mol/mol). The persisting small foramen ovale is followed by cardiologists.

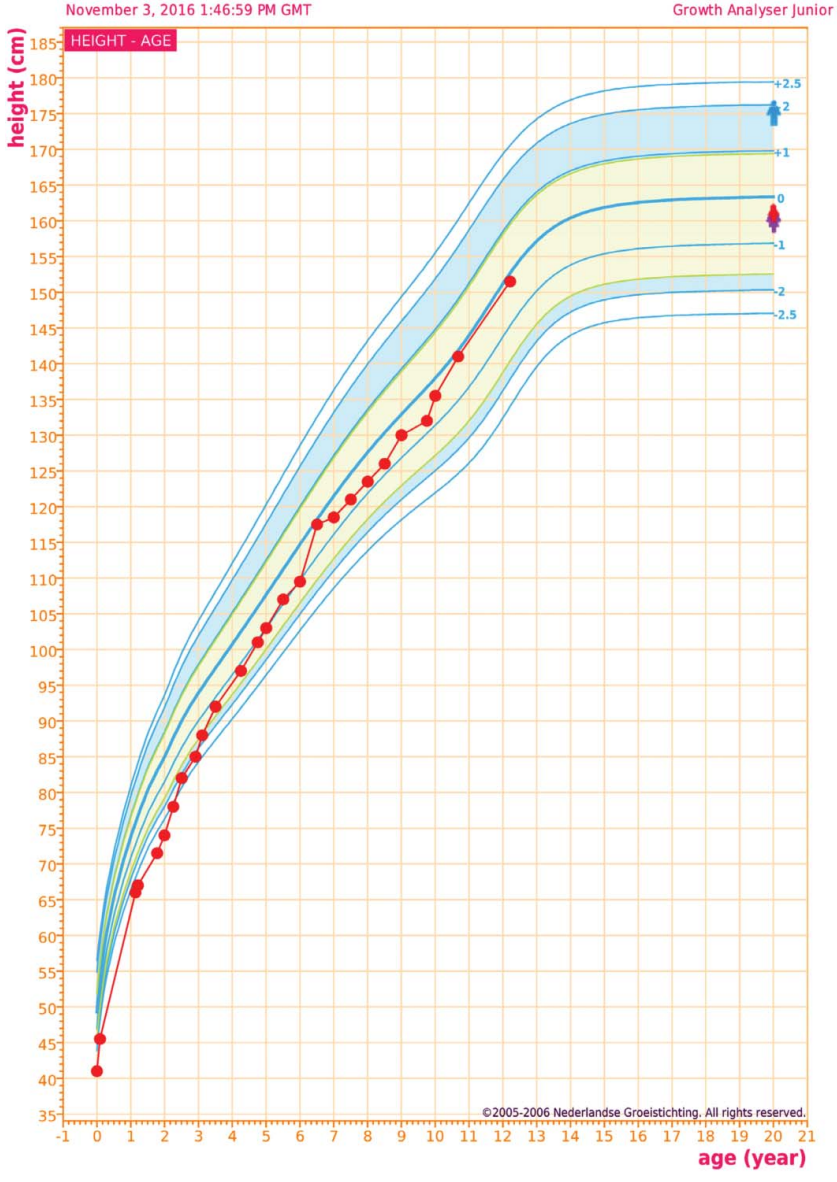

Figure 1. Growth chart of the girl since birth.

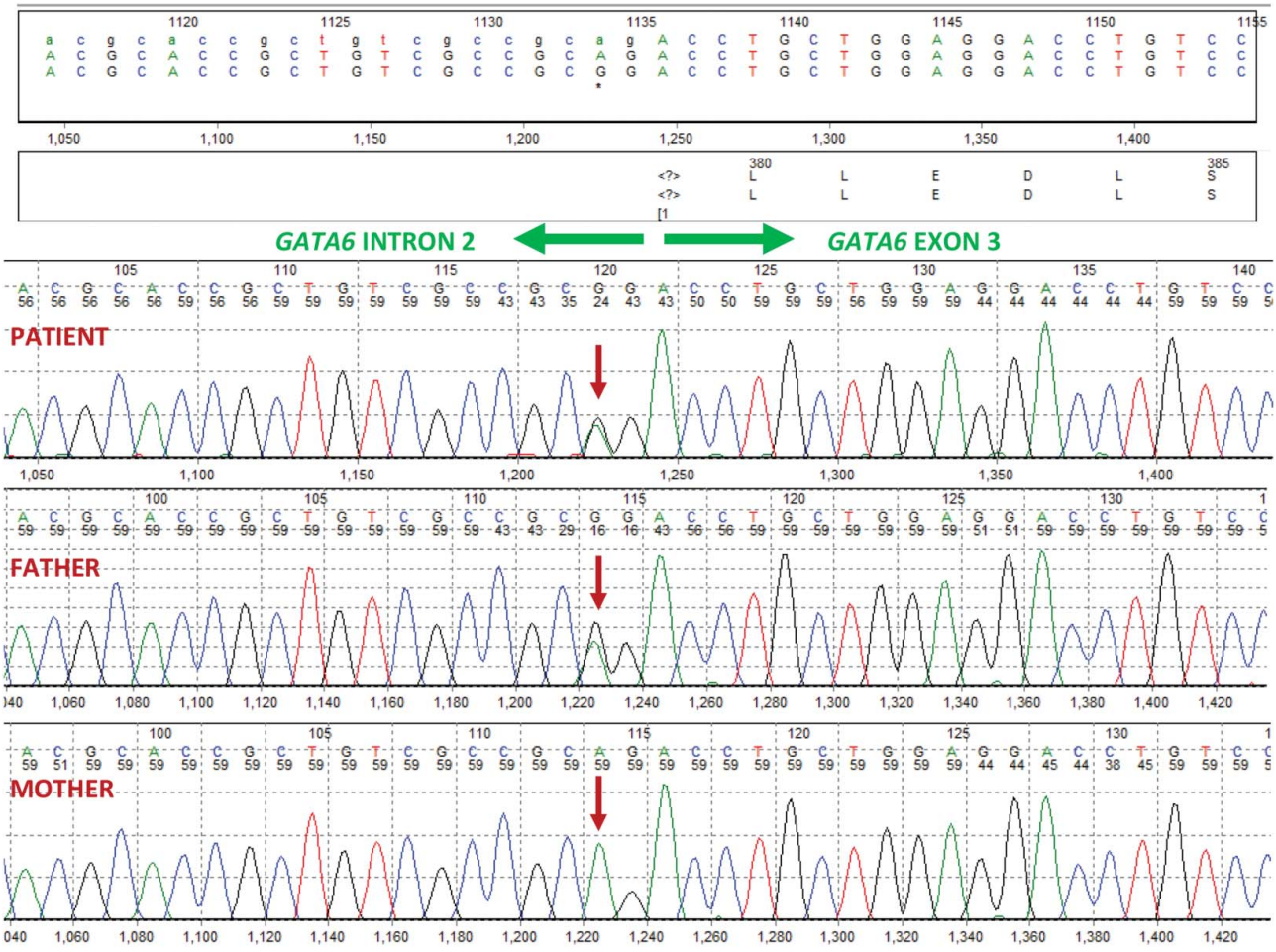

Figure 2. Elecropherograms showing the presence of the *GATA6* splicing mutation in the proband and her father.

Genetic testing

Agenesis of pancreas had not been suspected up to 2 years of age because of detectable C-peptide and no clear signs of exocrine pancreatic insufficiency. A first series of DNA analyses excluded mutations in the *KCNJ11*, *ABCC8* and *INS* genes as well as biallelic mutations in *GCK*. After MRI at 4 years of age, *PDX1* and *PTF1A* gene mutations were excluded as well.

Further analysis revealed a heterozygous *GATA6* splicing mutation, c.1136-2A>G. This mutation affects the conserved splice acceptor site of intron 2 (c.1136-2A>G) and is predicted to cause aberrant splicing (Figure 2).

Testing of parental samples revealed that the mutation had been inherited from her father; therefore, very likely to cause congenital heart defect as well as diabetes in both patients. The mutation was not found in the DNA sample from the paternal grandparents of the girl, indicating that it has arisen *de novo* in the father.

Thus, in the family examined in this study, the autosomal dominant transmission of a *GATA6* mutation is associated with a congenital heart defect (PDA) in the two relatives, pancreatic agenesis and PNDM in the daughter and pancreatic hypoplasia and juvenile-onset diabetes in the father. In a cohort of 795 neonatal diabetes patients, 24 (3%) have been reported to have *GATA6* mutations, 21 of them with complete absence or marked hypoplasia of the pancreas, including our patient [16]. The most frequent extra-pancreatic features among the patients with *GATA6* mutations are congenital heart defects (83%), but additional extra-pancreatic features are reported, too. McMillan et al. [17] report a case of neonatal diabetes and pancreatic hypoplasia resulting from a *de novo* mutation of the *GATA6* gene and several associated anomalies including truncus arteriosus, gallbladder agenesis, an inguinal hernia and protein-losing enteropathy. This illustrates the variable phenotype associated with mutations of this gene [17,18]. Some of the patients have late onset diabetes, but others have isolated cardiac defects only [16].

Our familial case of *GATA6* haploinsufficiency presents the main clinical characteristics of the syndrome: diabetes and cardiac defect [15–21]. Among familial cases, variable penetrance of the diabetes phenotype has been reported regarding age of onset, severity of diabetes

and exocrine insufficiency, whilst the cardiac phenotype is highly penetrant [16,22,23]. The family described here highlights the variable clinical presentation of the diabetic phenotype, with pancreatic agenesis and PNDM in the proband and hypoplasia, juvenile onset diabetes and subclinical exocrine insufficiency in the father. It is of interest to discuss if the hypoplastic 3.5 mm pancreas initially existed in the girl, being subjected to apoptosis later on in the absence of GATA6 supportive transcription factor. The same low levels of C-peptide and fecal elastase in the daughter and in the father do not reflect real pancreatic endocrine and exocrine functional capacity in each patient. The proband is our first case with PNDM out of 4150 children with diabetes registered in the Clinic of Diabetes at the University Pediatric Hospital – Sofia since 1970, including five children with TNDM.

Conclusions

Genetic analysis is important in patients with neonatal or late onset syndromic diabetes. Identification of the genetic defect guides treatment and allows prognosis, assessment of recurrence risk and prenatal diagnosis. Identification of a *GATA6* mutation in the family described here explains the cause of the syndromic neonatal and juvenile onset diabetes, although the mechanism underlying the phenotypic variability of the disease is still unknown. *GATA 6* mutations could be suspected at any age beyond infancy in diabetic patients with inherited cardiac anomaly like in the father.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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